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The effect of buffer solution choice on protein adsorption and lubrication

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ABSTRACT

In vitro buffers are frequently used to formulate model synovial fluids to investigate the role of individual constituents in synovial joint lubrication. This work examines how buffer choice affects protein film formation in static and rolling conditions. Solution pH dominates both the protein adsorption kinetics and the formation of tribofilms in static and rolling conditions respectively. Under static conditions, equilibrium adsorbed protein films from all buffers tested have similar properties although three distinct modes of adsorption, governed by the pH of the buffer, are observed. Films formed under rolling conditions are also pH dependent, with large irregular deposits formed in solutions with pH 7.4 or lower. The adsorption properties of proteins under static conditions only partially govern the lubrication properties of proteins.

1. Introduction

Synovial fluid (SF), is a complex aqueous suspension, which contains numerous proteins, proteoglycans, lipids and hyaluronic acid. The exact composition varies from patient to patient, making a '*master*' solution hard to define. For healthy SF the pH ranges from between 7.3 and 7.4 and the total protein content is 18–20 mg ml^{-1} [1]. In the diseased state the pH and protein concentration can increase up to 8.4 [2] and 34 mg ml^{-1}, respectively [1]. It is very difficult to collect and store adequate volumes of SF required to perform tribological investigations. Hence, model SF lubricants are employed. Bovine calf serum (BCS) is commonly used as a test lubricant [3] as it is readily available, has similar pH and salt levels to SF, and can be diluted to achieve a similar overall protein concentration as SF. Despite this, BCS has been shown to not closely simulate the lubrication of SF in either friction or wear studies [1,3,4]. Further disadvantages of BCS are the variation from batch to batch in its constituents, and the different protein composition to synovial fluid. Another approach is to prepare model fluids using a known concentration of a SF component in a buffer. This allows the roles of individual SF components to be studied in a systematic manner. It also presents the possibility of preparing model fluids which more closely match the variation in fluids from patient to patient.

Proteins, as the main constituent of both SF and BCS, are commonly considered as molecules that may improve joint lubrication. Protein solutions have been shown to reduce friction and wear in several studies [5–8], commonly attributed to an 'adsorbed' protein film. Buffers are used when preparing protein solutions to control the pH and ionic strength of the solution. The choice of buffer is at the discretion of the investigator, and a range of buffers are used by different laboratories. However, the effect of the buffer composition on protein film formation is generally not acknowledged. Protein adsorption is a complicated phenomenon, influenced by many factors, such as solution pH [9,10], ionic concentration [11], protein concentration [12], hydrophobic interactions [13] and protein conformation [7]. As buffers vary in chemistry, pH and ionic strength, the choice of buffer will impact on the protein adsorption to test surfaces, including the rate of adsorption, total adsorbed amounts, reversibility of adsorption, and the properties of an adsorbed layer. This calls into question the validity of comparing adsorbed protein films in tests using different buffers, where the effects of buffer composition cannot be distinguished from other factors. The role of buffers on protein adsorption requires clarification.

In this work a distinction will be made between adsorbed and deposited layers. Adsorbed layers are proteins layers formed on a surface whose nature is similar to those formed under equilibrium conditions in a static solution. These layers are composed of proteins and hydrated ions only. Deposited layers are observed on a surface following dynamic testing. Due to the tribological conditions, these deposited layers can have very different chemical and physical properties to those adsorbed films formed using the same lubricant. There is no consensus on the existence, nature, and the effects of the protein films deposited on joint lubrication. Deposited protein films have been described as 'solid-like' layers,

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gels or viscous surface layers. 'Solid-like' layers strongly adhere to the surface and are highly immobile. This type of layer has been associated with a reduction in metal-on-metal wear [3,8]. One should note that these solid like layers have only been observed *ex-situ* when the layers have been dried. As protein layers have been observed to alter in strength and surface adherence dependent on hydration [7,14,15], the properties of dried surface layers should only be tentatively linked to in-situ layers.

Gel layers differ from 'solid-like' layers as they are highly hydrated, but they still adhere strongly to rubbing surfaces [7,8,16]. Denatured albumin which is less hydrated than native albumin has been shown to preferentially adsorb on hydrophobic surfaces [7,12,17]. Widmer et al. [14] and Heuberger et al. [7] surmise that adsorbed, hydrated protein gel films give lower friction coefficients than more solid-like films composed of compact, heat-denatured, unfolded albumin based on their work with UHMWPE and denatured proteins. In work on artificial cartilage, Murakami et al. [18] have directly observed that adsorbed protein gel layers in a contact are resistant to sliding and reduce friction. However, the contact pressures exerted by artificial cartilage are much lower than that from artificial joints, and the material properties, in particular the structure and the hydration of artificial cartilage is quite different to implant materials. Gel surface layers were observed by Myant et al. with a point contact between a femoral head and a glass disc as shown in [11]. A protein enriched film was formed at the inlet and was carried through the contact. These films were thicker than statically adsorbed films reaching thicknesses greater than 100 nm. The films were easily disrupted by reversal in flow direction and removed by surface scratches. This suggests weak surface adhesion, particularly when compared to dried deposits. However, once damaged or removed these films rapidly recovered to >100 nm within a few seconds.

This lack of consensus across the field on the nature of protein film lubrication may be due to the complex behaviour of proteins, which in turn can be dependent on how the protein solutions are prepared and the buffer used. The adsorption of proteins on surfaces is frequently investigated under a static, equilibrated condition, which can be very different to the conditions in a tribological contact. It is unknown whether the behaviour of protein adsorption observed under static conditions can be used to predict wear rates and friction of rubbing surfaces immersed in protein solutions during dynamic processes. Mechanisms other than protein surface adsorption may be more significant in governing the success of protein in facilitating joint lubrication. In this work the role of model synovial fluid buffers in protein lubrication is investigated. In particular, we examine which aspect of these buffers (pH, ionic strength or buffer molecule) has the dominant effect in protein surface adsorption. Tribological tests are then used to determine the role of protein surface adsorption in film formation under rolling conditions.

Two key questions were asked:

1. What is the effect of buffer composition on protein adsorption and EHL film formation?
2. Are static adsorption measurements relevant to the lubrication process?

Albumin was chosen as it is the predominant protein in synovial fluid. The static adsorption [19,20] and tribological characteristics [7,21–23] of albumin have been widely studied, but no work to date examines the relationship between these behaviours and the impact of buffer choice. In this study the adsorption properties of different albumin/buffer solutions were measured using a quartz crystal microbalance (QCM). The results were compared to film formation under rolling lubrication conditions for a ball-on-flat contact. Whilst this is not a direct simulation of an artificial joint, the test conditions are chosen to be close to those experienced in a metal on metal (CoCrMo) hip prosthesis. The test speed is 10 mm s^{-1}, which is in the normal operating range for the hip joint [24]. The mean contact pressure used in this study is 200 MPa, which is higher than that experienced by correctly operating joints (<100 MPa [21]). However, pressures in this range do occur in small diameter hip joints with large clearance [21], or under edge loading [25].

2. Materials and methods

The adsorption properties of bovine serum albumin (BSA) under static condition in a selection of model synovial fluid buffers are investigated with QCM. The effect of buffer composition on lubricant film thickness formed under continuous rolling was then examined by optical interferometry for a glass/CoCrMo interface.

2.1. Solutions

Eight buffer solutions are prepared as listed in Table 1, all of which have been used in published work on protein lubrication in synovial joints [4,22,26–28]. Di-ionised water, with pH ~5.6, is used as the simplest buffer, with no adjustments to pH or additional salt. To test the effect of ionic strength, buffers are prepared at an ionic strength of ≤ 10 mM or physiological ionic strength of 154 mM. The pH of the buffers is also varied to investigate the effect of pH on protein buffers lubrication, with water and saline used with no pH adjustment (pH ~5.6),

Table 1
The composition, pH, ionic strength and the theoretical Debye screening length for buffer solutions used in this studied.

Buffer	Buffer concentration (mM)	Components	pH	Ionic strength (mM)	Debye screening length (nm)
Water	0	Water	5.6	0	961
Saline pH 5.8	0	NaCl, water	5.8	154	0.78
Saline pH 7.4	0	NaCl, NaOH, water	7.4	154	0.78
Phosphate buffered saline	10	Na_2HPO_4, KH_2PO_4, KCl, NaCl, water	7.4	154	0.78
Tris buffered saline	10	2-Amino-2-(Hydroxyl-methyl)-1,3-propanediol, NaCl, water	7.4	154	0.78
HEPES buffered saline	**10**	**4-(2-Hydroxyethyl)piperazine-1-ethane-sulfonic acid, NaCl, Water**	7.4	154	0.78
Tris	10	2-Amino-2-(hydroxyl-methyl)-1,3-propanediol	8.1	5	4.30
Tris buffered saline pH 8.1	10	2-Amino-2-(hydroxyl-methyl)-1,3-propanediol, NaCl, water	8.1	154	0.78